Two Transmissible Plasmids in *Rhizobium leguminosarum* strain 300

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The transposon Tn5, which specifies kanamycin resistance, was inserted into transmissible plasmids of *Rhizobium leguminosarum* strain 1062, a derivative of strain 300. In this way several kanamycin-resistant derivatives of the two smallest plasmids (pRL8JI and pIJ1001) were obtained, but there was no evidence that any of the other plasmids of strain 1062 was transmissible. Kanamycin-sensitive derivatives, each apparently cured of either pRL8JI or pIJ1001, still induced nitrogen-fixing nodules on peas and were not phenotypically distinct from the parental strain. Both plasmids were transmissible at low frequency to other *R. leguminosarum* strains, but they could be mobilized efficiently by pRL1J1, another transmissible *R. leguminosarum* plasmid. When Tn5-marked pIJ1001 was transferred to a strain of *R. phaseoli*, the majority of the transconjugants lost the ability to nodulate *Phaseolus* beans, the normal host for this species. This was due to the loss from *R. phaseoli* of a nodulation plasmid which was apparently incompatible with pIJ1001.

**INTRODUCTION**

Large plasmids, greater than 100 megadaltons (Md), appear to be ubiquitous in strains of the genus *Rhizobium*: typically one strain contains several different plasmids (Nuti et al., 1977; Casse et al., 1979; Gross et al., 1979; Hirsch et al., 1980; Beynon et al., 1980). In some strains, plasmids carry genes necessary for the establishment of nitrogen-fixing nodules on the roots of legumes (Johnston et al., 1978a; Zurkowski & Lorkiewicz, 1979; Brewin et al., 1980a, b, c; Hirsch et al., 1980; Beynon et al., 1980). However, it is still not clear whether all the plasmids found in *Rhizobium* strains have a role in nodulation and what other functions might be determined by such plasmids.

*Rhizobium leguminosarum* strain 300 has been investigated in this laboratory for several years. A circular chromosomal linkage map has been established (Beringer et al., 1978b). Strain 300 contains at least six large plasmids (Hirsch et al., 1980), one of which (pRL10J1) carries genes concerned with nodulation and nitrogen fixation (Hirsch et al., 1980; Buchanan-Wollaston et al., 1980; Hombrecher et al., 1981).

Here we describe properties of the two smallest plasmids (pRL7J1 and pRL8J1) in strain 300. Both are transmissible, but neither appears to be essential for nodulation of peas or nitrogen fixation.

**METHODS**

*Strains of bacteria.* These are shown in Table 1.

*Culture conditions and media.* These were as described by Beringer (1974).

*Plant tests.* Peas (var. Wisconsin Perfection) or *Phaseolus* beans (var. Fino) were inoculated, grown and assayed for nodulation as described by Beynon et al. (1980).

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### Table 1. Bacterial strains

<table>
<thead>
<tr>
<th>Species</th>
<th>Relevant characteristics</th>
<th>Source/Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>Rhizobium leguminosarum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>Wild-type isolate and parent of all <em>R. leguminosarum</em> auxotrophic mutants below</td>
<td>Johnston &amp; Beringer (1975)</td>
</tr>
<tr>
<td>1062</td>
<td><em>ura</em>-14 <em>trp</em>-16 <em>str</em>-86 (contains pIJ1001)</td>
<td>Buchanan-Wollaston et al. (1980)</td>
</tr>
<tr>
<td>6015</td>
<td><em>phe</em>-1 <em>trp</em>-12 <em>rif</em>-392 <em>str</em>-37 <em>nod</em>-6007 (contains pIJ1000)</td>
<td>Johnston et al. (1978a)</td>
</tr>
<tr>
<td>2482</td>
<td><em>phe</em>-1 <em>ade</em>-27 <em>rif</em>-65 pRL1JI</td>
<td>This paper</td>
</tr>
<tr>
<td>KH70</td>
<td>1062 pIJ1001::Tn5</td>
<td>This paper</td>
</tr>
<tr>
<td>KH151</td>
<td>1062 pIJ1001::Tn5</td>
<td>This paper</td>
</tr>
<tr>
<td>KH81</td>
<td>1062 pRL8JI::Tn5</td>
<td>This paper</td>
</tr>
<tr>
<td>KH129</td>
<td>1062 pRL8JI::Tn5</td>
<td>This paper</td>
</tr>
<tr>
<td>KH169</td>
<td>1062 pRL8JI::Tn5</td>
<td>This paper</td>
</tr>
<tr>
<td>KH223</td>
<td>KH129 pRL1JI</td>
<td>This paper</td>
</tr>
<tr>
<td>KH224</td>
<td>KH151 pRL1JI</td>
<td>This paper</td>
</tr>
<tr>
<td>3855</td>
<td><em>str</em>-279 mutant of <em>R. leguminosarum</em> field isolate 128C53</td>
<td>This paper and Brewin et al. (1980c)</td>
</tr>
<tr>
<td><strong>Rhizobium phaseoli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4292</td>
<td><em>rif</em>-398 mutant of <em>R. phaseoli</em> field isolate 8002</td>
<td>This paper</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1830</td>
<td><em>pro</em> met <em>nal</em> pJB4JI</td>
<td>Beringer et al. (1978a)</td>
</tr>
<tr>
<td>1895</td>
<td>Strain RR1 carrying the internal HindIII fragment of Tn5 in pBR322</td>
<td>J. Shine &amp; B. Rolfe</td>
</tr>
</tbody>
</table>

**Bacterial crosses.** Filter matings and patch matings were done as described by Jacob et al. (1976) and by Johnston et al. (1978b), respectively.

**Bacteriocin production.** This was tested as described by Hirsch (1979).

**Melanin production.** Patches were made on complete (TY) medium supplemented with L-tyrosine (30 μg ml⁻¹) and CuSO₄·5H₂O (10 μg ml⁻¹). Both these supplements were found to enhance melanin production. Plates were incubated at 28 °C for 3 d and were then placed at room temperature for a further week before being scored.

**Isolation of Rhizobium plasmids.** Plasmids were isolated and analysed on agarose gels by the method of Hirsch et al. (1980).

**Hybridization of labelled probe DNA to Rhizobium plasmids.** Rhizobium plasmids, separated by electrophoresis on agarose gels, were transferred to nitrocellulose filters according to the method of Hombrecher et al. (1981). The probe DNA, which was a derivative of pBR322 into which an internal HindIII fragment of the transposon Tn5 had been cloned, was constructed by J. Shine. The *Escherichia coli* strain containing the Tn5-probe was kindly provided by B. Rolfe and this strain was used as a source of plasmid DNA which was prepared essentially as described by Clewell & Helinski (1969). Nick translation of the plasmid was done according to the method of Rigby et al. (1977). Hybridization of the radioactively labelled probe to the *Rhizobium* plasmids on nitrocellulose filters, and the conditions for washing the filters and for autoradiography, were as described by Hombrecher et al. (1981).

**RESULTS**

Our criteria for plasmid nomenclature are as follows: plasmids found in field isolates of *R. leguminosarum* have the prefix 'pRL' and the suffix 'JI'; plasmids constructed in this laboratory have the prefix 'pIJ'.

In strain 300, the fastest migrating plasmid band actually comprises two comigrating plasmids (pRL7JI and pRL8JI); in strain 1062, a nodulating (Nod⁺), nitrogen-fixing (Fix⁺) derivative of strain 300, these plasmids have been resolved by a spontaneous deletion in pRL7JI (Hirsch et al., 1980) producing the plasmid pIJ1001. The evidence for this conclusion is as follows. Firstly, in strain 300 the fastest migrating band stains more brightly than do either of the two small plasmid bands in strain 1062 (Hirsch et al., 1980). Secondly, when radioactively labelled DNA corresponding to pIJ1001 was used as a hybridization
Transmissible plasmids in Rhizobium

Table 2. Transfer of kan from derivatives of strain 1062 to strain 6015

<table>
<thead>
<tr>
<th>Donor strain</th>
<th>Approximate frequency of kan transfer to strain 6015</th>
<th>Plasmid into which Tn5 had been inserted</th>
<th>Designation of Tn5-marked plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH70</td>
<td>$10^{-8}$</td>
<td>pIJ1001</td>
<td>pIJ1011</td>
</tr>
<tr>
<td>KH151</td>
<td>$10^{-8}$</td>
<td>pIJ1001</td>
<td>pIJ1012</td>
</tr>
<tr>
<td>KH81</td>
<td>$10^{-6}$</td>
<td>pRL8JI</td>
<td>pIJ1013</td>
</tr>
<tr>
<td>KH129</td>
<td>$10^{-6}$</td>
<td>pRL8JI</td>
<td>pIJ1014</td>
</tr>
<tr>
<td>KH169</td>
<td>$10^{-6}$</td>
<td>pRL8JI</td>
<td>pIJ1015</td>
</tr>
<tr>
<td>KH223 (=KH129 pRL1JI)</td>
<td>$10^{-4}$</td>
<td>pRL8JI</td>
<td>pIJ1014</td>
</tr>
<tr>
<td>KH224 (=KH151 pRL1JI)</td>
<td>$10^{-3}$</td>
<td>pIJ1001</td>
<td>pIJ1012</td>
</tr>
</tbody>
</table>

probe with plasmids from strains 1062 and 300 which had been transferred from gels to nitrocellulose filters, it was found that in strain 300 the hybridization was specifically to the fastest migrating band and in strain 1062 the probe hybridized to the deleted plasmid pIJ1001 and not to pRL8JI (P. R. Hirsch, personal communication; see Fig. 1). Thus, by using strain 1062 we hoped to distinguish physically between Tn5 insertions into either of the two small plasmids of strain 300 derivatives. The nomenclature and approximate sizes of the plasmids in strains 300 and 1062 are shown in Fig. 1.

Insertion of the transposon Tn5 into plasmids

In order to facilitate the identification and manipulation of particular plasmids in strain 1062 we attempted to mark at least some of them by insertion of the kanamycin resistance transposon Tn5. This was done by mating strain 1062 with Escherichia coli strain 1830 (containing the Tn5-carrying plasmid pJB4JI which cannot replicate in Rhizobium) with selection for the transfer of kan to strain 1062; the progeny have Tn5 transposed into the Rhizobium genome (Beringer et al., 1978a). The frequency of kanamycin-resistant derivatives was about $10^{-6}$, i.e. some hundred times higher than the frequency of kan mutants in strain 1062. Two hundred separate progeny colonies were purified. Strains in which Tn5 was inserted into transmissible plasmids were identified by using each of the 200 progeny as potential donors in crosses with strain 6015 [a non-nodulating (Nod−) derivative of strain 300 whose plasmids are shown in Fig. 1] with selection for transfer of kan. Five of the strains...
Fig. 2. Plasmids of strains KH70 (Tn5 in smallest plasmid) and KH129 (Tn5 in second smallest plasmid) and some kanamycin-sensitive derivatives of these strains, after agarose gel electrophoresis. Track 1, strain KH70; tracks 2, 3 and 4, three kanamycin-sensitive derivatives of KH70 showing, respectively, a deletion, a loss and a rearrangement of pIJ1011; track 5, a kanamycin-sensitive derivative of KH129 showing the loss of pIJ1014; track 6, strain KH129; track 7, *E. coli* strain 1843 containing the plasmid pJB3J1 (37 Md).

donated *kan* at frequencies at least ten times higher than the background. These fell into two groups (Table 2), with transfer frequencies of about $10^{-8}$ (KH70 and KH151: class I) and about $10^{-6}$ (KH81, KH129 and KH169: class II). All five strains produced nitrogen-fixing nodules on peas identical to those of the 1062 parent.

Plasmids from the five strains were examined on agarose gels. Those of the class II strains were indistinguishable from the parental strain 1062. The plasmids of one such strain (KH129) are shown in Fig. 2, track 6. In both class I strains (e.g. KH70, shown in Fig. 2, track 1) the fastest migrating plasmid was slightly larger than the corresponding one in strain 1062. Thus, in KH70 the two smallest plasmids are separated by a smaller distance than they are in strain KH129.

When the plasmids of each of these five strains were transferred to nitrocellulose paper, and radioactively labelled pBR322::Tn5 DNA was used as a hybridization probe, hybridization was specifically to the smallest plasmid of class I and to the second smallest plasmid of class II strains. Thus, the class I and class II strains had Tn5 inserted into pIJ1001 and pRL8J1, respectively. It is not clear why the insertion of Tn5 into pIJ1001, but not into pRL8J1, led to a detectable change in its mobility.

*Deletions and loss of Tn5-marked plasmids*

In an attempt to identify the phenotypes determined by pIJ1001 and pRL8J1, we isolated kanamycin-sensitive derivatives of each of the five strains; some such derivatives should have lost part or all of the Tn5-marked plasmids.
Table 3. Isolation of kanamycin-sensitive derivatives from KH strains

<table>
<thead>
<tr>
<th>Parental strain</th>
<th>No. of colonies examined</th>
<th>No. of kanamycin-sensitive colonies</th>
<th>Status of Tn5-marked plasmid compared with KH parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH70</td>
<td>5000</td>
<td>7</td>
<td>Unchanged 1 Lost 2 Deletion 1 Other 0</td>
</tr>
<tr>
<td>KH151</td>
<td>8000</td>
<td>1</td>
<td>1 0 0 0</td>
</tr>
<tr>
<td>KH81</td>
<td>2500</td>
<td>8</td>
<td>0 8 0 0</td>
</tr>
<tr>
<td>KH129</td>
<td>1200</td>
<td>7</td>
<td>0 7 0 0</td>
</tr>
<tr>
<td>KH169</td>
<td>3300</td>
<td>2</td>
<td>1 1 0 0</td>
</tr>
</tbody>
</table>

Cultures of each of the strains were grown in the absence of kanamycin and plated on complete medium. Plates carrying about 100 colonies were replicated to complete medium containing kanamycin to identify kanamycin-sensitive colonies. The frequencies of such derivatives ranged from $10^{-3}$ to $10^{-2}$ (Table 3).

The plasmids of all the kanamycin-sensitive derivatives were examined on agarose gels (Table 3 and Fig. 2). In some cases they were indistinguishable from the corresponding parental strain, indicating that only a very small region in or near Tn5 was removed or that perhaps the kan gene in Tn5 had suffered a point mutation. Other derivatives appeared to have lost the Tn5-marked plasmid completely with no ‘new’ band being apparent. Such cured derivatives of strains KH70 and KH129 are shown Fig. 2, tracks 3 and 5, respectively. In the case of KH70 two of the sensitive derivatives had suffered deletions in pIJ1011, the Tn5-marked derivative of pIJ1001 (Table 2). Figure 2, track 2 shows the plasmids of such a strain in which approximately 30 Md had been lost from the original plasmid. In the other deletion, approximately 40 Md had been lost.

One other derivative of strain KH70, termed KH213G, was more complex (Fig. 2, track 4). Although KH70 had a Tn5 insertion into pIJ1001, the derivative KH213G had lost the bands corresponding both to pIJ1001 and to pRL8JI, and three new bands had appeared with approximate sizes of 160, 130 and 40 Md. Moreover, the bands corresponding to the two smallest of these ‘new’ plasmids were faint compared with the other bands, indicating that they may not have been present in all cells in the population. To test this, seven separate colonies were purified and analysed. Four of these subclones had plasmid patterns that were the same as that of the original culture, KH213G. In two clones, the 160 Md plasmid was absent but the two smaller plasmid bands were retained and these two bands stained brightly. In the seventh clone, both the 160 Md and the 40 Md plasmids were retained and stained brightly. We suppose that in the original KH213G culture the 160 Md plasmid was present in most cells but gave rise (by intramolecular recombination) to derivatives containing both the 130 Md and the 40 Md plasmids or derivatives containing only one of these smaller plasmids. We do not know if the sum of the molecular masses of the two smaller plasmids equals that of the supposed parental plasmid. The origin of the 160 Md plasmid is not clear. Since KH213G has lost both the pRL8JI and the pIJ1011 bands, one possibility is that these two plasmids recombined to form the 160 Md plasmid and that during this process Tn5 was lost.

All of the kanamycin-sensitive derivatives tested nodulated peas and fixed nitrogen normally, and bacteria isolated from the nodules were still kanamycin-sensitive. The fact that strains which had apparently lost pRL8JI or pIJ1001 were symbiotically normal indicates that neither carries genes that are essential for nodulation. Indeed, there was no obvious difference between these cured strains and their parents with regard to growth rate, nutritional requirement or colony morphology.

Transfer of Tn5-marked plasmids to R. leguminosarum strains 3855 and 6015

Strain 3855 has two plasmids of about 200 Md and no plasmids that migrate in the region of pRL8JI or pIJ1001 (Brewin et al., 1980c). When kan was transferred from strains KH70 or
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KH151 to strain 3855, a new band appeared corresponding to pIJ1001. Similarly, the kan transconjugants obtained from matings with strains KH81, KH129 or KH169 had an extra band corresponding to pRL8JI. In no case did either of the strain 3855 resident plasmids disappear.

We also examined the plasmids of derivatives of strain 6015 which had received kan from each of the five KH donor strains. Figure 1 shows that in strain 6015 itself pRL7JI and pRL8JI comigrate and pRL10JI has suffered a deletion to yield plasmid pIJ1000. When strains KH81, KH129 and KH169 were used as donors, the transconjugants were indistinguishable from strain 6015 (two transconjugants were examined from each cross). The kan transconjugants derived from the cross of strain 6015 with KH151 were more varied. Of the seven examined only one had the pattern that might have been expected, i.e. there was a band corresponding to pIJ1012 in this strain and the fastest migrating band in strain 6015 had become less bright. This is consistent with pRL7JI having been replaced by pIJ1012. The other six transconjugants had lost the fastest migrating band (containing pRL7JI and pRL8JI) and had gained a new plasmid which in five cases was about 160 Md in size and in one case was 150 Md. One possibility is that these 'new' plasmids arose by recombination between pRL8JI and the Tn5-marked pIJ1012 and that this recombinant plasmid is incompatible with both pRL7JI and pRL8JI in strain 6015.

Mobilization of Tn5-marked plasmids by pRL1JI

pRL1JI is a 130 Md plasmid which was detected in field isolate 248 of R. leguminosarum. It is transmissible to other Rhizobium strains at high frequency (about $10^{-2}$) and carries genes that determine nodulation and nitrogen fixation and the production of a bacteriocin of medium molecular weight (Hirsch, 1979; Johnston et al., 1978a; Hirsch et al., 1980; Hombrecher et al., 1981). In crosses between a derivative of R. leguminosarum strain 300 which harboured pRL1JI and a strain of R. phaseoli, Beynon et al. (1980) obtained evidence suggesting that pRL1JI could mobilize pRL8JI and pIJ1001.

To verify this, we transferred pRL1JI into strains KH129 and KH151 (representatives of classes II and I, respectively) by mating these strains with strain 2482 (a derivative of strain 300 carrying pRL1JI) and examining recipients for their ability to make the pRL1JI-specified bacteriocin. The pRL1JI derivatives of KH129 and KH151 were termed, respectively, KH223 and KH224. The plasmids of KH223 and KH224 were as expected; there was a band corresponding to pRL1JI in addition to the bands of KH129 and KH151, respectively.

These two pRL1JI strains were used as donors in crosses with strain 6015. The frequencies of kan transfer observed were much higher than when KH129 or KH151 were used as parents (Table 2), the factors of increase due to the presence of pRL1JI in KH223 and KH224 being, respectively, about $10^2$ and about $10^4$.

From each cross, 40 transconjugants were tested for pRL1JI-specified bacteriocin production; in both cases 75% of the kan transconjugants had acquired bacteriocinogenicity, i.e. there was an approximately 75-fold enrichment for the inheritance of pRL1JI among the kan transconjugants. The plasmids of a sample of the kan transconjugants from both crosses which either lacked or contained pRL1JI (as judged by their production of bacteriocin) were examined on gels.

The kan transconjugants that had not coinherited pRL1JI following mating with strain KH223 were indistinguishable from strain 6015 itself, but with KH224 (just as with KH151) the transconjugants had acquired a new plasmid of about 160 Md and had lost the fastest migrating band of strain 6015 (two examined from each cross). The kan transconjugants that arose from the cross between strain KH223 and strain 6015 and which had co-inherited pRL1JI-determined bacteriocinogenicity had a band corresponding to pRL1JI but otherwise were the same as strain 6015. The three transconjugants that made the pRL1JI-specified bacteriocin and had inherited kan from KH224 had acquired bands corresponding to pRL1JI and to pIJ1012. Thus co-transfer with pRL1JI apparently favours the inheritance of pIJ1012 as an intact entity. The mobilization of pIJ1012 by pRL1JI was apparently not due simply to
the presence of a high frequency transfer plasmid \textit{per se} since derivatives of KH151 into which the P1 group plasmid pJB3JI (Brewin et al., 1980a) had been introduced had an enhanced frequency of \textit{kan} transfer but the factor of increase was about $10^5$ compared with the increase of about $10^8$ mediated by pRL1JI.

\textit{Transfer of pIJ1012 and pIJ1014 to \textit{R. phaseoli}}

Beynon et al. (1980) found that, following the transfer of a derivative of pRL1JI (termed pJB5JI) to \textit{R. phaseoli} strain 1233, about 1\% of the transconjugants coinherited pIJ1001, the smallest plasmid of strain 1062. When this happened, the smaller of the two plasmids (pRP1JI) present in the recipient was lost and these transconjugants lost the ability to nodulate \textit{Phaseolus} beans and to make melanin, indicating that both these characters are determined by pRP1JI. (Melanin production is characteristic of strains of \textit{R. phaseoli} but not \textit{R. leguminosarum}.) Since pIJ1001 itself is transmissible it was of interest to see if the transfer of the Tn5-marked derivatives of it would also lead to the loss of \textit{Phaseolus} nodulation ability.

We crossed strain KH151 with \textit{R. phaseoli} strain 4292, a rif derivative of a field isolate different from the strain 1233 used by Beynon et al. (1980) but which, like strain 1233, contains two large plasmids (about 190 and about 220 Md). The frequency of \textit{kan} transconjugants was about $10^{-6}$, and all of eight transconjugants tested produced melanin. To allow further opportunity for plasmid segregation, four single colony derivatives from each of the original eight transconjugants were retested for melanin production. All four subclones derived from five of the original transconjugants were white. The subclones from the other three patches were heterogeneous with, respectively, one, two and three of the four subclones being black and the rest white. All the black subclones nodulated \textit{Phaseolus} beans and the nodules fixed nitrogen at normal rates, whereas none of the white subclones nodulated.

We examined the plasmids of a sample of the transconjugants on gels. All the white subclones were identical; they had gained a plasmid band corresponding to pIJ1012 and had lost the smaller of the two resident plasmids (termed pRP2JI) of strain 4292. The two black transconjugants that were examined had also lost pRP2JI but did not acquire a band corresponding to pIJ1012.

We interpret this result to mean that pIJ1012 and pRP2JI are in the same incompatibility group and that following the transfer of pIJ1012 into strain 4292, the incoming plasmid recombined with pRP2JI to form a plasmid too large to be seen on gels. We assume that in the black transconjugants this recombinant plasmid is retained but that it is unstable and may reconstitute pIJ1012, and during this disassociation the part of the plasmid corresponding to pRP2JI is lost, and that this loss renders the strain unable to make melanin or to nodulate \textit{Phaseolus} beans.

\textit{Transfer of pIJ1012 to other field isolates of \textit{R. leguminosarum}}

In view of the high frequency loss of nodulation following the transfer of pIJ1012 from strain KH151 to \textit{R. phaseoli}, it was of interest to see if non-nodulating derivatives of \textit{R. leguminosarum} field isolates could be generated in a similar way. We therefore crossed KH151 with 16 prototrophic \textit{R. leguminosarum} field isolates (into all of which a rif mutation had previously been introduced) and selected for \textit{kan} transfer. For ten of these recipients no transconjugants were detected (frequency $< 10^{-8}$), but with the other six, \textit{kan} derivatives arose at frequencies ranging from $10^{-8}$ to $10^{-6}$ depending on the recipient. One transconjugant from each of the six successful crosses were assayed on peas. In all cases the peas were nodulated and fixed nitrogen as well as did those inoculated with the parental field isolate, indicating that in none of these cases had a ‘pea nodulation plasmid’ been eliminated.

\textbf{Discussion}

We have obtained independent insertions of Tn5 into each of the two smallest plasmids (pIJ1001 and pRL8JI) in \textit{R. leguminosarum} strain 1062, a derivative of the field isolate strain
300. This result suggests that these two are the only plasmids of strain 300 that are transmissible. By isolating kanamycin-sensitive derivatives of these strains we obtained strains in which pIJ1001 or pRL8JI appeared to have been lost, with no obvious change in phenotype, symbiotic or otherwise; thus the only phenotype known to be determined by these plasmids is transmissibility. However, we have not excluded the possibility that in these 'cured' derivatives some of the plasmid sequences are still present, as would occur, for example, if part of the plasmids had integrated into the chromosome.

One of the kanamycin-sensitive derivatives of strain KH70 (in which Tn5 was in pIJ1001) appeared to have arisen via recombination between pRL8JI and the Tn5-marked pIJ1001 to form an unstable recombinant. It is perhaps surprising that such a recombinant occurred since P. R. Hirsch (personal communication) failed to find homology between pIJ1001 and pRL8JI. It is possible that the proposed recombination does not depend upon DNA homology, or that if it does, the sequences are too short to be detected by the techniques used.

When Tn5-marked derivatives of pIJ1001 were transferred to *R. leguminosarum* strain 6015 the plasmid profiles of the transconjugants again suggested that pIJ1001 and pRL8JI may recombine, since in most cases the transconjugants had lost both pRL8JI and pRL7JI (this latter being the plasmid from which pIJ1001 was derived), these probably being replaced by a recombinant plasmid.

Transfer of pIJ1012 to *R. phaseoli* strain 4292 also appeared to be associated with recombination between this pIJ1001 derivative and one of the resident plasmids. In this case the proposed recombinant plasmid involved pIJ1012 and the smaller of the plasmids (termed pRP2JI) of strain 4292, but derivatives of this recombinant arose at high frequency in which pIJ1012 appeared to be reconstituted and the pRP2JI sequences were lost. The loss of pRP2JI was associated with the loss of melanin production and of the ability to nodulate *Phaseolus*. Beynon et al. (1980) found that in a different *R. phaseoli* strain these phenotypes were also determined by a small plasmid and, furthermore, that it was lost following the transfer of pIJ1001 into *R. phaseoli*. It will be of interest to see how widespread among strains of *R. phaseoli* is the loss of nodulation ability and melanin production following the introduction of pIJ1001. At least in the sample of *R. leguminosarum* strains that we tested, transfer of Tn5-marked pIJ1001 did not generate transconjugants unable to nodulate peas.

The interactions of pIJ1001 with the nodulation plasmid of *R. phaseoli* strains 1233 and 4292 are somewhat similar to the interactions of pVW3JI with pRL6JI, the nodulation plasmid of 128C53 (Brewin et al., 1980c). In both cases a transmissible plasmid which does not determine nodulation ability belongs to the same incompatibility group as a nodulation plasmid, and in both cases recombinants between the two plasmids arise. However the pIJ1001/pRP2JI recombinants appear to be more unstable than the pVW3JI/pRL6JI plasmids.

A highly transmissible *R. leguminosarum* plasmid (pRL1JI) efficiently mobilized Tn5-marked derivatives of pRL8JI and pIJ1001, the factor of increase being particularly high (105) in the latter case. It was striking that when pRL1JI and pIJ1001 were cotransferred to strain 6015 the transconjugants appeared to have inherited pRL1JI and the Tn5-marked pIJ1001 intact. It is not clear why pRL1JI results in the inheritance of an intact pIJ1001 in contrast to the case where strain KH151 itself is the donor.

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